

Application No. 10/590,118  
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Attorney Docket No. 4544-062454

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application No. : 10/590,118 Confirmation No. : 2820  
Applicants : Prakash Singh Bisen et al.  
Filed : June 11, 2007  
Title : DIAGNOSTIC KIT FOR DETECTING PULMONARY  
AND EXTRA PULMONARY TUBERCULOSIS  
Group Art Unit : 1645  
Examiner : Rodney P. Swartz, Ph.D.  
Customer No. : 28289

Mail Stop Amendment  
Commissioner for Patents  
P. O. Box 1450  
Alexandria, VA 22313-1450

SECOND REVISED AMENDMENT

Sir:

In response to the Office Action dated January 14, 2008 and the Notice of January 6, 2009, Applicants submit the following amendments and remarks.

**Amendments to the Specification** begin on page 2 of this paper.

**Amendments to the Claims** begin on page 13 of this paper.

**Remarks** begin on page 17 of this paper.

I hereby certify that this correspondence is being electronically  
submitted to the United States Patent and Trademark Office on  
February 6, 2009.

02/06/2009

Date

Signature

Mary Ann Mulvihill

Typed Name of Person Signing Certificate

deproteinized with one fifth volume of 0.7% of KCl. To the organic layer so obtained, washing was performed with 3:48:47 of ~~Chloroform~~chloroform: methanol: water. Moisture was removed by using benzene. Solvent was evaporated with the aid of rotary vacuum evaporator ~~with the aid of rotary vacuum evaporator~~ and a dried film of lipid was obtained. Neutral lipids were removed as described above with chilled acetone. Empty weight of round bottom flask was taken ( $W_a$ ). ~~Flask~~The flask was weighed along with the dried lipid film. 37.5 g of crude lipid was isolated. The crude product was further purified by silica gel H chromatography and purified PC was characterized by Thin thin layer chromatography and PC estimation was performed as known in the prior art (Sunamoto, J. et al. 1978)(19).

**Please replace the paragraphs beginning on page 5, line 19 and continuing on page 6, line 11, with the following rewritten paragraphs:**

-- ~~1 mg of Mycobacterium tuberculosis pellet was taken after centrifugation of mycobacterium tuberculosis pellet was taken after centrifugation of mycobacterial growth in Sauton's medium. The pellet was washed twice with 1X PBS to get rid of media remnants. The pellet was then suspended in 4 ml of 1X Phosphate buffered saline. 4-8 acid washed beads of 5 mm diameter was added to the above. The sample was vigorously shaken for 10 mm on a vortex. The suspension obtained was mixed with an equal volume of Freund's Incomplete Adjuvant. The mixture was squeezed through 22g needle repeatedly till it reaches a desired level. 100- $\mu$ l of suspension was inoculated to a young rabbit of 2-8 months. The mixture of purified glycolipid antigens (1-2mg) in PBS (pH7.2) were emulsified with an equal volume (1.0-2.0ml) of Freund's Incomplete Adjuvant (IFA) and immunized to (2-8 months old) young rabbit subcutaneously (100-500 $\mu$ l/rabbit) and boosted in similar manner after 15 days interval thrice and titer was monitored (1:60-1:120) periodically. A number of rabbits were inoculated in the same manner.~~

-- Rabbits were bled after one month after the third booster and serum was obtained. The reactivity of serum was checked with the liposome antigen suspension as described in the test procedure given in next paragraph. ~~T<sub>1</sub> and the~~ reactivity titer was checked. A booster administration of the antigen (50-100-500  $\mu$ l) was again repeated. An enhanced titer of about